WHITE MOUNTAIN RESEARCH STATION

UNIVERSITY OF CALIFORNIA, BERKELEY

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OBJECTIVE

To establish physiological base line data, and to develop physiological procedures and instrumentation necessary for the automatic measurement of hemodynamic and metabolic parameters during prolonged periods of weightlessness.

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I. BIOCHEMISTRY

A. *Elemental Analyses of Biological Materials.*

It is our objective to develop a method whereby biological samples such as urine, feces, feed, tissues, etc., can be analyzed in a uniform manner, accurately, reproducibly and with the least number of steps, for the nine elements: nitrogen, phosphorus, sulfur, chlorine, sodium, potassium, calcium, magnesium and iron. Such an approach is urgently needed for studying metabolic function in animals during their growth stage, their adult maintenance stage, or when they are subjected to various experimental conditions.

Classes of material which must be analyzed include:

1) The diet -- "The input"

2) Feces)
) "The output"
3) Urine)

4) The total animal body composition.

The "input, output" analyses are normally used in metabolic balance studies in many laboratories. However there exists considerable diversity from one laboratory to another (a) in sample preparation for analysis, depending on the type of biological material, and (b) in the methods used for quantification of the individual elements.

It is for these reasons that a uniform procedure was developed and tested in this laboratory.

Following are the details and discussions of the various steps leading towards the quantitative determination of the nine elements:

1. Freeze drying. All materials, whether initially homogeneous such as urine or liquid diet, or coarse mixtures such as feces and inclusions

(of known origin and amount), various dry foods and animal carcasses, are initially freeze dried. The freeze drier, equipped with four stainless steel trays was a Virtis model USM-15 with a condenser capacity of about 12 liters.

The samples were weighed and then spread uniformly onto stainless steel trays coated with a sheet of polyethylene. The maximum loading of the trays was not allowed to exceed 7-8 liters of sublimed water. About 72 hours were required to completely dry the material under these conditions. Immediately after opening the freeze-drier, the dried material was collected by lifting the polyethylene sheet from the tray and securing the edges together with masking tape. The total bundle was then weighed. The contents from the sheet were then carefully transferred into a wide-mouth glass jar of suitable volume equipped with a teflon lined screwcap. By weighing the polyethylene sheet with all the masking tape, the net dry matter was determined by difference. In addition, by knowing the weight of the original material the water content was also assessed.

2. Fat extraction. This part of the procedure was carried out in a well ventilated fume hood. Sufficient petroleum ether (boiling range 30°-60°C) was added to the glass jar to cover the freeze dried contents, but occupying no more than 80% of the volume. The contents were shaken and left standing for 24 hours. The extract was then carefully decanted into a second jar of the same volume. Fresh petroleum ether was added once more to the first jar, capped, shaken and again left standing for 24 hours. During the second period of extraction, the first extract was allowed to evaporate. It was estimated that the first extraction resulted in the removal of 90% or more of the fat content. By combining the decanted petroleum ether from the second period of extraction with that remaining from the first period, both jars were left in

the fume hood to evaporate to dryness. The evaporation could be speeded up by placing the jars in 60°-80°C water bath. By carefully weighing the extraction jar before adding the petroleum ether and after drying the extract, the net fat content can be obtained.

3. Homogenization. The moisture and fat-free matter was then passed through a Wiley mill. The result was a fine dry powder. The latter was stored in a well capped glass jar.

4. Kjeldahl digestion. About 5 to 10 minutes before weighing samples for the sulfuric acid digest, the sample jars were shaken vigorously for several minutes. The "dust" was allowed to settle before opening the jar. Appropriate samples were weighed on an analytical balance. Precautions were taken to avoid air turbulence in the room. Digestion was carried out in a routine manner as previously described in publications from this laboratory. Subsequently the digests were diluted to 550 ml with distilled water to a precalibrated mark on the Kjeldahl flask.

5. Alkaline ashing. Chlorine and sulfur cannot be determined in the Kjeldahl digest. Chlorine evaporates during heating as hydrochloric acid, and sulfur cannot be measured in a sulfuric acid medium. To avoid this inherent difficulty with chloride and sulfur, a second sample was subjected to alkaline ashing. In this manner the elements were preserved as non-volatile compounds, which were readily acid soluble.

A weighed, dry sample was placed in an aluminum oxide crucible (Coors AD 999, 100 ml capacity) and a solution of potassium carbonate was added. The amount of potassium carbonate added was roughly equal to the tenth of that of dry sample by weight. Excess potassium carbonate caused charring which was difficult to burn off. The solution of potassium carbonate was added carefully onto the wall of the crucible and allowed to wet the whole

sample. Mixing was accomplished by slight agitation of the crucible by hand.

The crucibles were then placed in a kiln, provided with a Foxboro thermostat and temperature control. The readings on the Foxboro thermostat were calibrated with a Cole-Parmer Dyna-Sense electronic pyrometer Model No. 8396. The temperature in the kiln was set at 120°C for 2 hours to allow all moisture to escape from the sample. Then the temperature was gradually raised by about 100°C each hour to a final ashing temperature of 450°C. After 4-6 hours the sample was completely ashed. It was convenient however to allow the ashing to proceed overnight.

Occasionally, black ash particles still remained, but repeated analyses for chloride showed no interference. After cooling, 10 ml of 1:6 analytical grade nitric acid solution was added to the crucible to dissolve the contents. The same 10 ml pipet was used to aspirate the solution and expel it slowly along the inside wall of the crucible in order to dissolve any material that might have spattered. This was repeated once or twice, and finally the solution was transferred into a 15 ml conical centrifuge tube. Any residual undissolved particles were forced to bottom by centrifugation, and the supernatant used for analysis.

6. Analysis. Some of the elements, namely calcium, magnesium, sodium, iron and phosphorus, could be analyzed in both the sulfuric acid solution of the Kjeldahl digest or in the nitric acid solution resulting from the alkaline ashing. This was actually done to check on the accuracy and repeatability resulting from the two sample decomposition methods. From these analyses it became apparent (1) iron is best analyzed in the alkaline ash rather than Kjeldahl digest because for the recommended sample size it is present in adequate concentration for measurement, and (2) calcium is also best analyzed in the nitric acid solution resulting from the alkaline ashing medium, because it solubilized more completely than in the

sulfuric acid medium of the Kjeldahl digest.

The methods employed for the quantitative estimation of the individual elements were previously developed in this laboratory and are the same as routinely used here, namely: 1) atomic absorption for calcium, magnesium, sodium, potassium and iron; 2) color development with subsequent spectrophotometry for nitrogen and phosphorus; 3) electrometric determination of chloride; and 4) nephelometric estimation of sulfur as sulfate. For reasons yet undetermined, difficulties have been encountered in the reproducibility of the sulfur analysis from the ashed samples.

An indirect method for the analysis of sulfur was also tried. It consisted of precipitating the sulfate with a known amount of barium, and measuring the excess barium (in the supernatant) by atomic absorption. All the inherent difficulties in the quantitative precipitation of barium were present. Linearity was obtained as a function of concentration versus atomic absorption readings; however, the sensitivity was extremely low.

Work will continue either to improve the procedure for sulfur using the light scattering characteristics of a suspension of barium sulfate, or to develop a new approach using a method which reduces sulfur to sulfide and determines the latter by electrometric microtitration.

To test the total procedure for the analysis of the nine elements in material of biological origin, a standard chemical mixture was prepared and tested. Table 1 indicates the composition of the standard chemical mixture. Table 2 shows the results of separate analyses for bovine liver, a standard mixture, and a combination of the two for a "recovery" study. The bovine liver was chosen as a standard reference material because it was prepared by the National Bureau of Standards and had a certified chemical composition for seven of the elements of interest. The recovery results shown in

Table 2 are as good or better than those obtained by the National Bureau of Standards.

The total method for all the elements except sulfur is now being utilized in the analyses of whole monkey carcasses which were ground, freeze dried, defatted and powdered.

B. *Microelectrophoresis.*

1. Technical improvements have been made for sample application to effect superior resolution.

2. Further studies of the fractionation of the isoenzymes of phosphoglucomutase have been carried out. It appears now that the supporting medium of choice is cellogel, because it will accept a larger sample about 4 times that usable with cellulose acetate. Thus, the minor bands in the electrophoretogram become visible. As a result, genetic typing of blood will be possible.

Lipoproteins. We are now able to perform lipoprotein fractionation and phenotyping on cellulose diacetate with Oil-Red O stain. Oil-Red O on cellulose triacetate previously used for the electrophoresis of plasma proteins was unsatisfactory for the specific differentiation of lipoproteins.

II. BIOINSTRUMENTATION

A. *Monkey Pod Restraint Subsystem.*

To provide optimal conditions within the restraint subsystem of the monkey pod several modifications have been made and tested with a monkey interface during this report period. Adjustments were made in the lower couch, jacket and the flexible waste seal.

The plastic couch referred to in Status Report No. 19 has replaced the stainless steel framed device used previously. To accommodate a greater range of morphological variation in the lower extremities stainless steel spacers have been incorporated laterally midway in the area of the upper and lower leg portions of the couch. These extenders provide a potential increase of 1, 2 or 3 cm in length from the minimum configuration.

Instead of attaching the jacket support to the couch by means of braided nylon cord as described in Status Report No. 20, 5 cm width Velcro strips have been secured to the periphery. Thus, individual support and monkey positioning within the couch can be made more readily and precisely.

The waist divider seal design and fabrication has utilized several types of materials. The multipieced design described in Status Report No. 18, involving latex rubber and dental dam was used to fashion several sizes to accommodate a series of monkeys in the adult male size range. In addition, mold forms have been made to the dimensions of the iliac-crest circumference and lower thoracic circumference. Spray-on silastic may then be used to fashion a one-piece divider seal, which appears to provide a better leak-proof arrangement with improved dermal compatibility. Initially, the silastic seals were made without a reinforced mat and some weakness developed in their structural integrity. With the incorporation of nylon tricot in the

fabrication of the seal its strength has been greatly improved. The silicone also has an advantage over the latex in its greater resistance to deterioration with time and the impact of biological fluids.

With the adjustability available in these portions of the restraint subsystem, 10 monkeys in a weight range of 9 to 12 kg were submitted to interface trials of 24-hour duration. The results of these trials further defined a set of conditions for the optimal configuration of restraint for each individual monkey.

B. *Pod Gas Metabolism Subsystem.*

The development and construction of a monkey pod, including an upper hood section for respiratory gas exchange measurements was described in Status Reports No. 18-20. In the gas metabolism subsystem, gas is drawn through the outlet port of the pod by a Metal Bellows Model MB-150 Pump with an operating range of approximately 5 to 15 liters per minute. The flow rate of the main stream from the pod is adjusted by means of a pinch-cock on a side-arm tube, leaking in a variable volume of make-up air just upstream from the pump, and downstream from the analyzer side-arm. Air flow rate through the outlet port of the pod is measured by an on-line Matheson Model 604 Flow Meter. Turbulence in the main stream is muffled by introducing a "windkessel" in the flow between the pod and the pump. Gas from the main flow is sampled through an analyzer side-arm by a Metal Bellows Model MB-21 Pump with an operating range of approximately 100 to 500 ml per minute. The flow rate in the analyzer line is adjusted by means of a needle valve upstream from the pump and measured by a Matheson Model 602 Flow Meter downstream from the pump. A two-way valve between the analyzer side-arm and the needle valve permits sampling of the main flow from the pod, or

alternatively, from a line leading to calibration gases and room air. The composition of a gas mixture is determined by passing the sample flow in series through a Cambridge Model 137-CS Aircraft Hygrometer System for water vapor content, a drying tube, a Beckman Model LB-1 Medical Gas Analyzer for carbon dioxide content, a Beckman Model F-3 Oxygen Analyzer for oxygen content, and a Medi-Science Model 205-AR Nitralyzer for nitrogen content. The entire array of analyzers, flow meters, pumps, valves, etc., are housed in an incubator box, 30 inches wide, 36 inches long, and 40 inches high, with a controlled temperature of 37°C, to enhance instrument stability and to prevent condensation of water vapor in the system lines. The signal outputs of the analyzers are recorded on a Brush Series 1707 Recorder.

The respiratory gas exchange of the animal is determined by the difference in composition between the incoming room air and the air leaving the pod, i.e., ΔF_{O_2} and ΔF_{CO_2}, and the flow rate of air through the pod. As a means of evaluating the reliability of the gas metabolism system for measuring oxygen consumption and carbon dioxide production, test runs were carried out by burning an alcohol lamp in the hood section of the pod. From the equation, $C_2H_5OH + 3\ O_2 \rightarrow 2\ CO_2 + 3\ H_2O$, the precise quantities of oxygen consumed and carbon dioxide produced can be computed by measuring the quantity of alcohol burned or consumed. The results of a series of such measurements are shown in Table 3 to indicate the accuracy and test-retest reliability of the system.

C. *Pod Lower Body Negative Pressure Subsystem.*

The design goals for LBNP instrumentation to be incorporated into the monkey pod were discussed in Status Report No. 20. Two requirements for this instrumentation were (1) adjustability of the waist seal to fit monkeys in the 8-13 kg weight range and (2) minimal additional restriction

of subject movement during periods when negative pressure is not applied.

The LBNP instrumentation fabricated during the last Status Report period was tested with the monkey pod and was found to satisfy the second, but not the first, requirement. The distance between the side rails of the pod restraint couch was then increased by about 4 cm, which allowed the use of a three-piece waist seal (in place of the former two-piece seal). Concurrently with the development of the modified waist seal, a one-piece divider seal was fabricated using a sprayable, silicone rubber (92-009 Dispersion Coating, Dow-Corning Corp., Midland, Michigan). This seal is graded in thickness from 9 to 18 mils. The central sleeve which contacts the monkey's abdomen is thin and highly flexible and the flat diaphragm portion is thicker and is reinforced with nylon tricot fabric inbedded in the silicone.

The three-piece waist seal and the silicone divider seal were tested with an uninstrumented monkey in the pod using the supporting LBNP instrumentation described in Status Report No. 17. A viscoelastic seat cushion described in Status Report No. 20 was used with the pod restraint couch for this test. The maximum pressure differential across the waist seal obtainable with this system was 90 torr. Several trials of 40 to 60 torr LBNP in the supine position were made with minimal discomfort for a monkey.

Two sizes of three-piece waist seals have been fabricated and three sizes of silicone divider seals are being fabricated which should accommodate most monkeys in the 8-13 kg weight range. With zero pressure differential across the LBNP waist seal, the monkey in the seated upright position can move about 1-1.5 cm in all directions perpendicular to the main body axis. Additional tests of 7 days duration will be made with the waist seal and silicone divider seal installed on a monkey to determine if these devices can be tolerated during long-term pod restraint. This evaluation will have

to be made prior to using the LBNP/pod system for studies of the effects of upright pod restraint on cardiovascular deconditioning (see Status Report No. 19). The development in this laboratory of an implantable telemetry device to monitor aortic blood pressure should provide an ideal method for obtaining heart rate and blood pressure measurements during LBNP/pod experiments.

D. *Pod Blood Pressure and Temperature Telemetry Subsystem.*

An implantable telemetry transmitter has been designed to transmit pressure and temperature information from an indwelling transducer to be inserted into the aorta of a monkey. The transducer consists of a 5 kohm piezoresistive bridge and a 6 megohm transistor, both housed in a shell 6 cm in diameter by 1-1/2 cm deep. Sensor leads pass from the aortic wall to the transmitter, which in turn will be attached to the apex of the pleural cavity. The transmitter measures approximately 5 cm by 2 cm by 1-1/2 cm.

The transmitter circuitry consists of two sub-carrier oscillators, one for temperature and the other for pressure, a main RF oscillator and a switching circuit alternating between pressure and temperature signals. Temperature information is transmitted for 20 seconds per cycle while pressure is transmitted for 4 seconds. This arrangement is necessary to reduce battery drain, since the 6 megohm temperature circuit drains much less battery power than the 5 kohm pressure bridge.

The receiving circuits have been completed and are presently under test. Implantation of the unit is planned after completion of bench tests and calibration.

E. *Construction and Initial Testing of a Six-Channel Temperature Telemetry System.*

A transmitter with six thermistor probes attached was designed to be implanted in a pig-tailed monkey for sequential transmission of temperatures from six different anatomical areas. The probes varied in length from 6 cm to 50 cm and were designed to reach the following organs: 1) psoas muscle, 2) kidney, 3) portal vein, 4) liver, 5) aorta, and 6) brain. The transmitter is to be attached to the psoas muscle just caudad to the kidney.

The transmitter circuit consists of 6 sub-carrier oscillators controlled by the 6 thermistor probes, a RF main oscillator, and a ring counter to switch the 6 thermistor-controlled circuits in sequence. Battery power is provided for approximately 1 year of operation. The transmitter exclusive of probes measured 8 cm by 1.7 cm.

The instrument was implanted in an adult male monkey but failed to function. After removal from the animal, it was determined that accidental damage to one of the probes during surgery permitted water to leak into the circuitry.

Another implantation with the repaired unit is scheduled for the near future.

F. *Silastic Urine Collection Device.*

A technique for the collection of the total uncontaminated urine output from a restrained monkey for a period of several days is currently under development in this laboratory. The collection device is a thin-walled silicone tube open at both ends and of sufficient internal diameter to be placed over the glans penis of an adult monkey.

The monkey penis has a relatively large glans to penile shaft

circumference ratio. In the initial model, a silicone ring was bonded to the proximal end of the tubing. Preliminary tests have shown that when ring is expanded and placed over the glans and allowed to retract on the penile shaft the silicone tube forms a relatively liquid tight conduit without restricting blood flow in the penis.

Some difficulties were encountered with the expansion of the ring when bonded directly to the tube, but a satisfactory solution was achieved when the tube was positioned approximately one centimeter past the glans and a properly sized expanded rubber ring was passed over the tube and allowed to contract against the shaft. This device was successfully used for a four-day urine collection from the couch restrained monkey, #379 Fitzwater. No tissue reaction was evident upon removal of the silicone tube.

G. *Adapter for CSF Catheter.*

To minimize the chances of bacterial invasion and still allow the withdrawal of clear cerebro-spinal fluid when needed, a device has been adapted to perform this function when placed on the distal end of an exteriorized catheter. The catheter is passed through the central aperture of a Clay-Adams adapter cap and heat flared. A threaded insert portion for the adapter was fabricated of 316 stainless steel with an increased diameter at the unthreaded end for the placement of a rubber serum vial stopper. A sterile 25 gauge disposable needle can then be aligned in the center of the cleansed rubber stopper, a puncture made and the saline filled catheter allowed to clear with the force of internal pressure and then a sample of CSF removed with a sterile syringe. Following sampling, the catheter is filled with sterile saline and the needle removed. The rubber serum vial stopper can be easily replaced at intervals following several samplings and before the beginning of physical deterioration of the rubber.

III. NUTRITION

A. *Monkey Nitrogen Requirement.*

Trials leading to the determination of the protein requirement of the pig-tailed monkey were concluded in April of 1972. The methodology utilized has been previously indicated in Status Report No. 20.

Table 4 contains the complete data from monkeys fed liquid diets providing intakes varying from 0 to 2.70 grams of nitrogen per day. Integumental losses, including all nitrogen losses except those from the urine, feces, and gases were determined and these values are shown in Table 5.

An apparent positive balance appears when the amount of nitrogen in the diet exceeds 1.14 g/N per day. This led to the consideration of the possibility that at levels higher than 1.14 g/N per day gaseous loss of nitrogen might take place through the lungs or other channels.

From all considerations a suggested optimum protein allowance for the pig-tailed monkey was estimated for both an ideal protein, such as whole egg, or a mixture of plant and animal protein with a biological value about 70%. These allowances are given in Table 6.

B. *Monkey Calcium Requirement.*

A preliminary protocol including a literature search and investigation of types of palatable ingredients suitable for definitive calcium balance trials with non-human primates has been formulated.

IV. PHYSIOLOGY

A. *Pod Metabolic Balance Tests.*

Several trials have been conducted to determine the metabolic effects of pod restraint on the pig-tailed monkey. An experimental protocol was established to encompass an 18-day trial in which the experimental subject was observed for 6 days in the cage, followed by a pod period of 6 days, and a post-pod cage period of 6 days. Body weight determinations were made on days 1, 6, 12 and 18. Twenty-four hour determinations of food and water consumption were made throughout the entire period. Daily feces and urine output were combined for each of the 6-day periods. This material was processed in a manner described in Status Report No. 20 for subsequent chemical analysis. The food and water consumption for 5 trials on 4 monkeys is shown in Table 7.

B. *Pod Gas Metabolism Tests.*

Following the construction and initial reliability tests of the monkey pod restraint and gas metabolism subsystem described under the Bioinstrumentation Section of this report, oxygen consumption and carbon dioxide production were measured on an adult male pig-tailed monkey, #314, Pompey. The experimental subject was placed in the lower and middle pod sections for 5 continuous days and allowed to consume food and water *ad libitum*. From 3 to 4 hours each day the upper pod was put on, and continuous measurements of the respiratory gas exchange were made during this period. Table 8 summarizes data obtained from this trial and includes measurement parameters which reflect some of the physical characteristics of the system.

C. *Monkey Temperature Tolerance.*

The experiment protocol for this study was described in Status Report No. 19. Rectal temperature and heart rate data at 6 of the 8 total air temperatures for monkey #378 were presented in Table 9 of Status Report No. 20. Before the series could be completed, this subject developed chronic diarrhea and anorexia and died within six weeks. The findings at autopsy did not give evidence supporting a connection between this animal's illness and participation in the temperature tolerance experiments.

Three additional monkeys were chosen for testing and subjected to the 8 air temperatures from 5° to 40°C at 50% relative humidity. Each monkey was initially tested at a neutral air temperature (25°C) from 1 to 4 times in an attempt to condition the animal to the total experiment protocol. Testing at the remaining air temperatures was begun for each subject when the monkey would sit in the restraint chair relatively calmly throughout the 2-hour control period. The sequence of air temperatures for a given monkey was chosen randomly.

A total of 3 tests with 2 monkeys at 35° and/or 40°C air temperature were terminated early due to extremely high rectal temperatures. In these cases the environmental chamber was set in the cooling mode, the chamber door opened and the subject monitored until rectal temperature approached the control value. All 4 subjects were able to complete the tests at the cold air temperatures.

Temperature tolerance in these tests was based on the following definition of thermal equilibrium. If a subject maintained a constant rectal temperature (0.1°C change, or less) during the last 80 minutes of the test then the subject was judged to be in thermal equilibrium with the environment and hence able to tolerate the environment. In this way

a maximum and minimum air temperature which could be tolerated was determined for each subject. Summary data showing final rectal temperatures for four subjects at air temperatures from 5° to 40°C are shown in Table 9. Data from monkey #378 mentioned above, is included, due to the fact that no signs of illness were present during the tests and there is no indication that the data for this subject differs from the data for the remaining 3 subjects.

The rectal temperatures at thermal equilibrium ranged from about 37.5° to 39.0°C. For the coldest temperatures at which each subject was in thermal equilibrium the rectal temperatures ranged from 37.4° to 37.5°C. This same range for three of the 4 subjects in the heat was 38.8° to 39.1°C. Subject #408, the exception in this case, was unable to maintain thermal equilibrium near 39°C. One contributory factor was this subject's tendency to struggle in the restraint chair after about 2 hours in the environmental chamber thus increasing heat production and rectal temperature. The final average control rectal temperature for the 30 tests with 4 subjects was 37.8°C. Thus 3 of the 4 subjects could tolerate about a 0.3°C drop in rectal temperature in the cold and a 1.2°C rise in the heat.

Thermal equilibrium was attained at a wide range of air temperatures in the cold (from 5° to 25°C) but 3 of the 4 subjects could tolerate at least 15°C. Three of the 4 subjects could tolerate 35°C as their maximum in the heat. Subject #405 was able to tolerate air temperatures of 5° to 35°C. This subject began shivering soon after entering the environmental chamber at 15°C and colder temperatures. The remaining 3 subjects were either not observed shivering or shivered only at the 5°C air temperature. This variability in shivering may account for much of the difference in their ability to tolerate cold.

None of the 4 subjects showed a transient rise in rectal temperature when moved from the control air temperature of 23°C to the environmental chamber at 5°C. The typical response in human subjects for this same air temperature change is a 0.3° to 0.5°C rectal temperature rise due to peripheral vasoconstriction. This suggests that the monkeys in this study were close to maximally vasoconstricted at the control air temperature. Pilo-erection was prevented in this study due to the rather close fitting nylon restraint jacket which was worn over the monkey's thorax and abdomen and was no doubt of little importance as a heat conservation mechanism. The general behavioral response to the cold seen in the 4 subjects was an attempt to "curl up in a ball", but since this was mostly prevented by the design of the restraint chair only closing of the hands and feet was possible. Two of the subjects increased their gross body movements at the coldest air temperature.

In the heat the monkeys tended to "go limp" and some increase in surface moisture was observed on the hands and feet. The skin on the rest of the body remained dry to the touch. Three of the 4 subjects increased their struggling in the restraint chair as rectal temperature increased. No panting was observed but one subject kept his mouth open during the latter part of a test at 40°C.

The heart rate response to air temperatures from 5° to 40°C for the 4 subjects is shown in Table 10. Each data point is the change in heart rate expressed as the final rate at a given air temperature minus the final rate during the corresponding control period at 23°C. It can be seen that the minimum change in heart rate occurred between 20° and 25°C. All 4 subjects showed an increasing heart rate with increasing air temperature above 25°C. Three of the 4 subjects showed an increased heart rate reaching

a maximum (about 30 beats/min) at a final rectal temperature of about 37.5°C. This was the lowest rectal temperature at which the subject group could maintain thermal equilibrium. Subject #377 did not show a heart rate increase in the cold. For this monkey there was a fairly direct relationship between heart rate and rectal temperature from 5° to 40°C air temperature. Of the remaining 3 subjects, #378 and #408 both had a decline in heart rate as rectal temperature decreased below 37.5°C at the 10° or 5°C air temperature.

Because the heart rate response in these tests was quite variable, especially in the cold, and because there were only 4 data points at a given air temperature it is somewhat premature to draw conclusions. The data do suggest, however, that as rectal temperature increases above the control value there is a corresponding increase in heart rate. This increase may be due to a direct temperature effect on chemical reaction rates causing an increase in metabolic rate, but the effect of the observed increase in body movement must also be a contributing factor. An increase in metabolic rate due to shivering or non-shivering thermogenesis as rectal temperature decreases below control values may produce the heart rate increase noted at rectal temperatures near 37.5°C. For rectal temperatures below that point the direct temperature effect on chemical reaction rates (including cardiac pacemaker tissue) may be the predominating factor in the observed heart rate decrease. The possibility of an increase in heart rate due to gross motor movement at the 5°C temperature must also be considered, especially for subjects #405 and #408.

All of the above temperature tolerance tests have been conducted at 50% relative humidity. In the future, tests at 25° to 40°C will be conducted at both low (20%) and high (80%) relative humidity. These tests should indicate the relative importance of evaporative cooling mechanisms in the

pig-tailed monkey. If evaporative cooling mechanisms are important in this species it would be expected that the upper limit of thermal equilibrium would be lower at 80% compared to 20% relative humidity due to the greater efficiency of these mechanisms at the lower humidity.

D. *Monkey Biorhythms.*

In Status Report No. 18 experiments were described in which monkeys were irradiated with a proton beam in an attempt to ablate the pineal gland. The lumbo-abdominal temperature (LAT) rhythms of these animals were monitored and the animals were subjected to two 180° L-L shifts of the light-dark cycle (L:D, 12:12) before and after head irradiation. Results of that study showed no effect of head irradiation on the time to shift the LAT rhythm 180°. Histological examination of at least two of the three irradiated animals has shown that the area of radiological damage did not include the pineal.

As the irradiation technique did not prove to be a useful method of pinealectomy, a surgical approach was made on monkey #364, Lysimachus. This proved (as described in the Experimental Surgery section of this report) to be a more successful method of removing pineal tissue. Histological examination of the removed tissue shows it to be pineal tissue, but whether there has been partial or complete pinealectomy will not be known until autopsy of the animal.

Two months after pinealectomy, a temperature radiotelemeter was surgically implanted retroperitoneally in the lumbo-abdominal region of monkey #364. Following suture removal, the monkey was placed in a room with two other monkeys who also had temperature implants. The room temperature was maintained at 25°C $\pm$ 1°C and fluorescent light on a timer circuit provided 12 hours of light and 12 hours of darkness.

LAT's were monitored continuously from the transmitter output, and were analyzed for rhythmic fluctuations by the level-crossing technique previously described in Status Report No. 18.

After a control period of 30 days to allow the LAT rhythms to become stable, the animals were subjected to three 180° L-L shifts with 29 days between each shift. With this length of interim period between shifts, LAT rhythms had stabilized for at least 2 weeks prior to the next phase shifts.

The results of these phase shifts are given in Table 11 which shows the number of days from the completion of the shift necessary for restabilization of the level-crossing point indicated. Four level-crossing points are considered in this analysis: the time at which the temperature has risen one mean-range quartile, the time at which the temperature has risen two mean-range quartiles (that is, has risen to the mean value), the time at which the temperature has fallen one mean-range quartile and the time at which the temperature has fallen two mean-range quartiles (that is, to the mean value for the day). Consideration of these 4 crossings gives an indication of how long it takes for the rising and falling portions of the temperature rhythm to adjust after a "zeitgeber" or environmental synchronizer phase shift.

From the data presented in Table 11 it can be noted that in most instances rising level-crossings require only one day to shift. This may be due to what Aschoff calls "masking", in which case the turning on of the lights is postulated to cause a transient rise in the LAT by an as yet undefined mechanism. This transient response may be superimposed on the underlying rhythm and, because the rise is a significant event relative to the daily mean, the level-crossing analysis results in a very rapid shift

of one of the rising crossing points. In only two cases do both rising crossings show this rapid shift. The rapid shift of the rise time was not seen in the previous phase shift study. The differences in phase-shifting time may be due to a difference in the level of illumination in the two rooms in which the studies were conducted.

Comparison of the data in Table 11 for the pinealectomized animal against the other unoperated control animals shows that in all cases the pinealectomized animal took longer for the LAT rhythms to shift, if one takes the longest time for any of the quartiles to represent the time for complete shifting of the LAT rhythm. In some cases this is only a matter of 1-2 days, which may not be significant with this analytical technique. Sequential phase shift trials presently in progress may help to clarify the existence of this phenomenon.

E. *Plasma Volume Studies Using Radioiodinated Serum Albumin and Fibrinogen.*

Although radioiodinated serum albumin (RISA) has been in routine use clinically for the measurement of plasma volume in man, and in this laboratory for pig-tailed monkeys, it is becoming evident that its use results in a small, but appreciable (5-10%), overestimation of the true plasma volume because of diffusion through the blood capillary walls. The use of the larger labeled fibrinogen molecule has been suggested to obviate this error. A study is presently in progress to test this suggested improvement, by the simultaneous measurements of plasma volume in a series of monkeys, using ^{131}I-labeled albumin and ^{125}I-labeled fibrinogen. The results for the first two animals in the series are shown below:

Animal No.	^{125}I-Fibrinogen Plasma Volume (ml)	^{131}I-Albumin Plasma Volume (ml)	Per Cent Difference*
2	362	374	3.3
328	314	330	5.1

* % Difference = [(Albumin Space - Fibrinogen Space)/Fibrinogen Space] 100.

F. *Measurement of Exchangeable Potassium in Pig-tailed Monkeys.*

The determination of exchangeable potassium by the isotope dilution technique using ^{42}K has been suggested as a measure of the actively metabolizing fraction of the body, i.e., the active protoplasmic cell mass, and is akin to the measurement of total body potassium by whole body scintillation counting. ^{42}K is injected into the subject intravenously and all of the urine is collected for the duration of the procedure. Following sufficient time for equilibration (1-2 days), a sample of urine is analyzed for ^{42}K by scintillation counting, and for total K by flame or atomic absorption spectrophotometry. Exchangeable potassium (K_e) is then computed from the relationship:

$$K_e = \frac{^{42}K \text{ injected} - {}^{42}K \text{ excreted (cumulative)}}{^{42}K \text{ specific activity in urine}}$$

It is clear from the above that total and complete collection of the urine is an important requirement, and particularly in the monkey, represents the most difficult technical problem.

After several trials at devising a suitable means for quantitative collection of urine from a pig-tailed monkey for 2-3 days, a satisfactory device and technique has been obtained. The description of this device is given under the Bioinstrumentation Section of this report. Simultaneous

measurements of exchangeable potassium by ^{42}K dilution, and total body potassium by whole body scintillation counting of ^{40}K, will be made in a series of monkeys to examine to relationship between the two parameters.

Although ^{42}K has a very slow equilibration time which makes for a cumbersome procedure, and may indeed never exchange completely with some small fraction of the body potassium pool, the measurement might be useful in situations where the cost and maintenance associated with whole body counters and the practicality of its operation under field conditions are limiting considerations. However, conclusions concerning the validity of the ^{42}K exchangeable potassium determination must await completion of the current study.

G. *Monkey Blood Oxygen Transport.*

Preparatory to a study on monkey blood oxygen transport, it was deemed necessary to study the effects of refrigeration of blood samples for periods up to six hours, because of the time required to complete the biochemical analyses involved. Venous plasma lactate, with an initial concentration of about 1.2 μmoles/ml plasma was found to increase by roughly 0.2 μmoles/ml plasma/hr. Venous blood pH, initially about 7.42, increased by about 0.01 pH unit/hr. Both the lactate and pH changes are statistically significant. Both arterial and venous P_{CO_2} appear to decrease slightly, while arterial and venous P_{O_2} appear to fall. Insufficient studies have been made on the P_{CO_2} and P_{O_2} to determine typical initial values, or to determine if the changes are significant.

H. *Monkey CSF Studies.*

Weekly 1.5 ml cerebrospinal fluid samples have been removed from a chronically catheterized pig-tailed monkey without deleterious effects for

a period of over 6 months. Analyses have been made of pH, cell count, glucose, chloride concentration and the cations of sodium, magnesium, potassium and calcium.

It is planned to implant catheters in strategic locations within the arterial and venous circulation to the brain in order to ascertain the biochemical and physiological changes occurring when the monkey is subjected to varying environmental conditions such as decreasing ambient air pressure.

I. *Growth in the Pig-tailed Monkey.*

During April and May anthropoidimetric and roentgenographic measurements were made on 9 pig-tailed monkeys of known age. While longitudinal data are still limited, 2 male pig-tailed monkeys have been observed from birth to 8 years of age, and extrapolated growth curves have been made on other male animals when sufficient sequential data was available. A summary of these data in relation to change in body mass is shown in Table 12. As previously indicated in Status Report No. 19, five phases of male growth can be defined by curve analysis as follows: I) Infancy; II) Juvenile Depression; III) Juvenile; IV) Adolescent Spurt; and V) Late Growth.

The appearance of ossification centers occur throughout these growth phases as noted in Table 13. Completion of epiphyseal fusion, however, takes place mostly in the late growth phase (V) as indicated by Table 14. Utilizing a skeletal maturation chart which is accepted for human clinical use, a comparison of these two types of male primates in regard to the time of bone formation as shown in Tables 15 and 16. The ratio of age of the human to the monkey when the ossification centers initially appear is quite variable. This variance is mainly due to the fact that many of the centers are present

at birth in the monkey but appear later in the human male. The time of completion of epiphyseal fusion is from 2 to 4 times earlier in the pig-tailed monkey than in man.

Similar growth data derived from female pig-tailed monkeys of known age was treated in the same manner as for the males and this information is shown in Tables 17-21. In contrast to male growth curve there is little or no evidence of an "adolescent spurt" and growth in body mass appears to proceed in a sigmoidal manner, as is the case in most species of the mammalian kingdom.

J. *The Use of a Proteolytic Enzyme in Maintenance of Chronically Implanted Vascular Catheters.*

Over a span of 10 years, progress has been made with procedures for maintaining the patency of chronically implanted vascular catheters. Improvements in experimental surgical techniques, in the use and preparation of materials which are more compatible with biological tissue, and in the post-surgical care of the animals are a few of the problem areas which have been discussed in previous status reports. Clotting has occurred in some catheters despite many precautions. Therefore, the use of proteolytic agents has been investigated. A preliminary report on Brinase, a fibrinolytic enzyme derived from the mold, *Aspergillus oryzae,* was indicated in Status Report No. 18. The use of this agent has been utilized in 2 vascular catheterized monkeys during this report period. In operation at the present time is a procedural schedule for patency checks on the catheters at 3- to 5-day intervals as follows:

1) The monkey is removed from his cage and placed in chair.

2) The nylon protective jacket is partially removed and the mid-section of the chair swabbed with 70% isopropyl alcohol.

3) Catheters removed from protective coin purse and distal valve ends placed on clean gauze pad.

4) Valve cap removed and placed in 2% Tincture of Iodine.

5) Open end of valve swabbed with 2% Tincture of Iodine.

6) Open end of valve cleansed with sterile 0.9% saline expressed from a 2 ml disposable syringe.

7) Syringe placed on open valve end, valve stem placed in open position and withdrawal of blood attempted.

8) If catheter patent, it is flushed with 0.9% saline, the catheter is filled with heparin saline solution at a concentration of 167 units/ml, and the valve is capped.

9) If blood does not flow readily from catheter after withdrawal, a slight injection is made followed quickly by withdrawal and the procedures of step 8 performed.

10) Finally, if step 9 is not sufficient to produce patency, Brinase in a concentration of 1,000 to 2,000 units/ml of 0.9% saline is injected into the catheter, the valve is capped and left until the next check.

The results of catheter patency checks on two pig-tailed monkeys from initial surgery up to and including the last check of this report period are shown in Tables 22 and 23. From these data it would appear that Brinase has been a helpful adjunct in maintaining the patency of chronically implanted vascular catheters particularly after 300 days post surgery.

No gross physiological changes can be attributed to the use of this agent as noted in the condition of the subject animals. Food and water intake and body mass were maintained.

K. *Physiological Studies of Seals.*

A study to determine whether the relatively large blood volume and red cell volume of a marine mammal preacclimatizes it to the hypoxia of high altitude was described in Status Report No. 20. Unfortunately, the preliminary results were difficult to interpret owing to a number of uncertainties, including the influence of growth and development on erythropoiesis in the harbor seal. Consequently, a final interpretation of the "seals at high altitude" experiment is being deferred until completion of a second study currently in progress.

Plasma volume, red cell volume, total body water, and certain hematological parameters are being measured in eight young harbor seals at the Physiological Research Laboratory, University of California at San Diego. The animals were all captured shortly after weaning, and measurements are being made at 6-week intervals to follow the body composition changes associated with growth and development of the harbor seal. An interesting facet of the work is that of determining the physiological significance of the deep diving experience during the normal development of an infant harbor seal. Half of the seals are being raised in a typical deep water tank, and the remainder in "dry" caging provided with a water sprinkler system, and without the opportunity for diving.

L. *Ion Binding Studies.*

Investigations of molecular mechanisms involved in calcification processes were continued with cation binding studies on subcellular fractions of developing bone tissue. Calvaria (average weight 0.14 g per calvarium) from 32 porcine fetuses measuring an average of 6.8 cm crown to rump length and 20.5 g in weight, were homogenized in 0.25 M sucrose solution and subfractionated by differential centrifugation.

Although many more calvaria were available in the present studies than in previous experiments, the yield of 17,000x*g* mitochondrial and 100,000x*g* microsomal (membrane) fractions was still insufficient to perform meaningful cation binding experiments. However, reasonable binding experiments were possible with the matrix fraction sedimenting at 600 g. Aliquots of matrix material washed 5 times with 0.25 M sucrose-100 mM citric acid medium (pH 5.0) were equilibrated in solutions containing 100 mM sodium, 100 mM potassium, 2 or 3 mM magnesium, 2 or 3 mM calcium and 0, 2 or 3 mM inorganic orthophosphate. Essentially no change in sodium or magnesium associated with the matrix was observed under these conditions but matrix potassium decreased by approximately 70% and matrix calcium approximately doubled. The presence of inorganic orthophosphate in the medium made no significant difference in the amount of calcium retained by the matrix under the conditions employed.

As in previous experiments on developing bone tissue, the observation of particular interest was the highly selective retention of sodium by repeatedly washed matrix material. Extracted matrix fraction treated identically as the other equilibrated samples but suspended in 0.25 M sucrose medium instead of in electrolyte solution, retained 0.36 mmol sodium, 0.09 mmol potassium, 0.07 mmol magnesium and 0.04 mmol calcium per gram of total matrix nitrogen. Although the amounts of unextractable ions differed somewhat from those found in previous experiments, nevertheless, sodium still remained in much higher amount than any other of the measured cations.

As one approach to checking the relative amounts of difficult to extract cations in matrix material, a total of 14 intact (unhomogenized) calvaria were digested by the Kjeldahl procedure and analyzed for cations. Two calvaria from fetuses of 9 cm length were carefully rinsed and digested

without extraction; 10 calvaria from fetuses averaging 9 cm length, and 2 calvaria from fetuses of 13.5 cm length were extracted extensively in several changes of cold citric acid solution prior to digestion and analysis. The average compositions of the calvania were as follows:

Calvaria	Number of Calvaria	Measured Cations (mmol/g nitrogen)					Total Nitrogen per Calvarium
		Sodium	Potassium	Magnesium	Calcium	Zinc	
Unextracted	2	1.041	0.067	1.658	59.508	0.037	2.200
Extracted	10	0.031	0.002	0.003	0.009	0.008	3.579
Extracted	2	0.183	0.008	0.009	0.034	0.002	7.950

The readily measurable sodium retained by the extracted calvaria makes up greater than 50% of the cations not extracted. This is especially significant in view of the very high amount of calcium present in the unextracted calvaria.

In addition to the 4 cations routinely measured, zinc was also determined in the calvaria because of increasing evidence in the literature of the importance of this metal ion not only in metabolism generally, but specifically in bone. A small but significant amount of zinc was found in unextracted calvaria, and smaller amounts were found in extracted calvaria.

Analyses of extracted whole calvaria and extracted matrix material from homogenized calvaria have both consistently demonstrated the preferential retention of sodium. In addition, as mentioned in a previous report (Status Report No. 20), evidence from our work and from that of other investigators suggests that silicon is also present in developing bone and may be involved in bone formation. Taken together, the available information strongly suggests the possible presence and importance of a sodium silicate component in developing

bone which is difficult to remove by acid extraction. In the study of molecular mechanisms involved in bone formation, it is of considerable interest and importance to investigate this phenomenon in depth.

The extraction experiments with intact calvaria have demonstrated that even moderately calcified calvaria can be readily decalcified by treatment with mildly acidic (pH 5) media. In view of the requirement for much more starting material for the subfractionation experiments than has been available to date, it appears feasible to acid extract calvaria prior to homogenization and thus increase the availability of usable calvaria. This approach may permit isolation of enough high speed fraction to perform more extensive and meaningful membrane cation binding experiments than has been possible to date.

Because of the primary importance of inorganic orthophosphate in biological systems generally and in calcification processes in particular, experiments were started to determine in detail the effect of inorganic orthophosphate on the binding of cations by cellular membrane fragments. As a preliminary survey, an experiment was set up to determine the concentration and pH dependences of the effect of phosphate ion on the simultaneous binding of sodium, potassium, magnesium and calcium by isolated rat liver cell microsomes. In the concentration dependence experiment, freshly isolated microsomal material was equilibrated in media containing 100 mM sodium, 100 mM potassium, 2 mM magnesium, 2 mM calcium and 0 to 20 mM inorganic orthophosphate at a constant pH of approximately 7.5. In the pH dependence study, microsomal material was equilibrated in media containing the given concentrations of sodium, potassium, magnesium and calcium plus 5 mM inorganic orthophosphate, and with pH varied from 3 to 9. Results to date indicate a selective two to threefold increase in bound calcium at both high phosphate concentration and high pH. Although a corresponding increase

in bound phosphate was not observed in these experiments, the selective binding of large amounts of calcium is of particular interest in the study of molecular mechanisms of calcification. However, further experiments are required to elucidate the mechanism by which this selective change in cation binding occurs.

Investigations of the role of heavy metals in ion adsorption and ion transport phenomena continued with studies of the binding of mercuric ion and its effect on sodium, potassium, magnesium and calcium retention by membrane materials. Previous experiments (Status Report Nos. 19 and 20) demonstrated that with increasing concentrations of mercuric ion in the equilibrating medium, bound mercury increased sharply and approached a saturation level of about 2.3 mmol/g N at 1.5 mM free mercuric ion concentration. Concomitantly, bound sodium and potassium were essentially completely displaced at 0.5 mM free mercury concentration, whereas bound magnesium and calcium decreased significantly at low mercury concentration but appeared to level off at higher mercury concentrations. A preliminary experiment designed to investigate the hydrogen ion dependence of mercury binding (Status Report No. 20) indicated a strongly pH dependent component of bound mercury and a pH independent component of approximately 1 mmol/g N.

Additional pH dependence studies with mercury have been carried out in which microsomal membranes were equilibrated in media containing 100 mM each of sodium and potassium, 2 mM each of magnesium and calcium and 3 mM mercuric ion, all as the chlorides, with equilibrium pH varied from 3.2 to 9.3. Under these conditions, bound sodium, potassium, magnesium and calcium were essentially zero at a pH less than 5.0 and gradually increased to levels of about 0.3 to 0.4 mmol each cation/g N at pH 9.3. Bound mercury, on the other hand, remained significantly above zero even at pH 3.2, being 1.26 mmol/g

N at this pH, decreasing slightly to 0.95 mmol/g N at pH 4.5 and increasing slightly to 1.1 mmol/g N at pH 6.0. Bound mercury thus reached a plateau at approximately 1.0 mmol/g N between pH 3.5 to 5.5, the binding being essentially independent of pH in this range. Between pH 5.5 and 7.5, however, bound mercury increased sharply from 1.0 mmol/g N to 2.3 mmol/g N at pH 7.5, and tended to level off at about 2.5 mmol/g N at pH 9.3.

The results indicate that there is a pH independent component of bound mercury of approximately 1.0 mmol/g N in the pH range of 3.2 to 5.5, and a strongly pH dependent component with a capacity of approximately 1.5 mmol/g N. The close correspondence between the capacity of the pH dependent component of bound mercury and the total capacity of about 1.5 mmol/g N for the alkali and alkaline earth metals suggests that mercury in this component may be bound to the same sites which bind sodium, potassium, magnesium and calcium. The nature of the pH independent component of bound mercury, however, requires further investigation. From the available information, it appears probable that mercury in this instance may be associated with the membrane in a non-ionic form such as organic mercury or elemental mercury, the latter form being reported to have a high solubility in lipid material.

V. EXPERIMENTAL SURGERY

A. *Chronic Indwelling Vascular Catheters.*

Three male adult pig-tailed monkeys still maintain patent vascular catheters at the end of this report period. Number 404, Tyrrel has had a functional pulmonary artery catheter for 551 days, while #399, Catesby has had aortic and pulmonary artery catheters patent for 433 days. Both of these animals were utilized for physiological studies on certain blood parameters, the results of which have been reported elsewhere in this and previous status reports. In addition, they have been semi-restrained in couches and chairs for periods of time varying from less than one hour up to 4 days duration.

Brinase, a fibrinolytic enzyme, has apparently helped to maintain the patency of these catheters and its use in this regard is reported in a separate section of this report.

Number 398, Ratcliff and #406, Herbert prepared with thoracic aorta catheters during the last report period were shipped to the Bishop Area on 12 February 1972 after the completion of base line trials at Berkeley, for studies of intermediary metabolism at high altitude. Unfortunately #398, Ratcliff at 246 days post surgery developed an acute left hemothorax and was sacrificed. Post-mortem examination revealed the catheter had been placed 1 millimeter farther into the lumen of the aorta than originally planned, and the distal tip had punctured the blood vessel wall.

Number 406, Herbert remains in good condition at the Barcroft Laboratory with a patent catheter (286 days post surgery).

No additional thoracic vascular catheterizations have been performed during this report period.

B. *Cisterna Magna Catheterization.*

On 16 February 1972, with improved techniques and the use of a stereotaxic head holder described in Status Report #20, a polyvinyl chloride catheter was surgically implanted into the cisterna magna via the foramen magnum of #296, Duncan. The catheter was placed in a 1:500 aqueous solution of benzalkonium chloride for one week prior to the scheduled surgery. Although the physical condition of the catheter was less flexible than originally anticipated, the insertion was accomplished and patency has been maintained for 161 days as of 31 July 1972. Approximately two months after surgery with some deterioration noted in the quality of the cerebrospinal fluid samples, Gentamicin$^{(R)}$ therapy was initiated. An intracatheter infusion of 0.8 ml of Gentamicin solution at a concentration of 1 mg per ml of 0.9 saline was made in addition to a 50 mg intramuscular injection of this same agent. This regimen was repeated for 6 days with the subject showing complete recovery. At this time a closed system adapter (described elsewhere in the report) was fitted to the distal end of the exteriorized catheter. Samples of clear CSF 1.5 ml in volume have been withdrawn at weekly intervals with no deleterious effects as evidenced by the monkey's maintenance of body weight, plus good general appearance and behavior.

C. *Pulpotomies.*

In order to improve the technique of canine teeth removal and maintain structural integrity of the mandible and maxilla of the adult male non-human primate a collaborative effort has been initiated with the University of California School of Dentistry. Under general anesthesia three monkeys, #19, Mercutio, #307, Angelo and #341, Philostrate, have had

their canine teeth amputated, the pulp cavity explored, debrided, irrigated and the root canal sealed. Two types of sealers were used. Sargenti formula (containing some toxic components in the form of paraformaldehyde, phenyl mercuric borate and lead oxide) or a calcium hydroxide - 9 amino acridine paste. All monkeys involved returned to a normal food and water intake following surgery. At a proposed minimum time lapse of three or four years these areas of the jaw would be available for macro and microanatomical evaluation.

D. *Multi-channel Telemetry Implant.*

A 6-channel temperature telemetry unit, whose development and bench testing were described in Status Report No. 20 and in another section of this current report was scheduled to be surgically implanted in two stages. On 14 June a laparotomy was performed on #380, Carlisle with thermistor leads being placed in the intestine, kidney, psoas muscle and liver with two remaining leads coiled subcutaneously for second stage surgical placement near the brain and ascending aorta. During this surgery there was some indication that a portion of the protective cover of the leads was disrupted. The subject made good progress through a recovery period. However, no electronic signal was detected from the implanted transmitter when the monkey was placed within an appropriately antennaed cage. Two weeks following the initial surgery the electronic package was removed from #380, Carlisle. On removal of the kidney lead the tip was seen to be separated from the balance of the shielding. Due to the possibility of fatal hemorrhaging with removal of the leads from the kidney and liver, the monkey was sacrificed.

E. *Single-Channel Body Temperature Telemetry Transmitters.*

During this report period temperature transmitters were surgically implanted retroperitoneally in the lumbo-abdominal region in three monkeys. The animals and dates of their surgery are as follows: #364, Lysimachus, 9 May 1972; #175, Salisbury, 30 May 1972; and #366, Thaliard, 31 May 1972.

One transmitter was removed from #377, Simple on 23 March 1972. As of 31 July 1972, a total of 6 monkeys are being maintained with this type of telemetry device.

F. *Pinealectomy.*

Previous attempts to ablate the epiphysis cerebri (pineal gland) by irradiation with a focused proton ion beam from the cyclotron had not produced the desired results. Thus, on 13 March 1972 direct surgical removal was carried out on adult male pig-tailed monkey, #364, Lysimachus. The procedure was accomplished with the aid of an operating microscope, and histological examination of the surgically removed tissue revealed that it was indeed that of the pineal gland. Post-surgical therapy consisted of the administration of 2 mg Dexamethosone per day. The subject was ataxic for 2 to 3 days, after which time recovery progressed rapidly. This monkey was subsequently implanted with a temperature transmitter on 9 May 1972.

VI. ANIMAL COLONY

A. *Lung Mite Screening.*

Although it is difficult to attribute the demise of non-human primates directly to infestation by lung mites, their presence at autopsy in the lungs of Asian type monkeys has been recognized. A reliable diagnostic technique that will detect carriers of this parasite is unavailable at the present time. In cooperation with Prof. Deane Furman, Chairman of the Parasitology Department, lung mite screening trials have been initiated. Pig-tailed monkeys of both sexes and varying ages were investigated. In general, this technique involved the induction of anesthesia with intramuscularly injected Ketamine(R) at appropriate dosage levels. A bronchial swabbing was made contacting as many secondary bronchi as possible in 2 to 3 minutes time. The swab was removed, examined, and a urethral catheter introduced into the same area where the swabbing has previously taken place. Five milliliters of physiological saline was injected into the catheter followed by aspiration of the saline into a collecting flask with the monkey held by the legs with the head down. The injection and aspiration were repeated. Lung mites were recovered in the aspirate from several of the monkeys. These particular monkeys are presently in quarters separated from the main colony. It is planned to repeat the tests on these monkeys and perform the same procedure on three monkeys from the main colony. If the repeated tests confirm the original diagnosis in regard to the number of mite larvae recovered, a chemotherapy trial will be conducted.

B. *Monkey Census.*

As shown in Table 24, at the beginning of this report period, the

monkey colony was composed of 56 pig-tailed monkeys and a total of 53 on 31 July 1972. One birth, a male #418, Robin, occurred on 19 March 1972. This offspring was sired by #51, Voltimand who was conceived and born in the colony, while the mother was an imported breeding female of unknown parentage. Four monkeys were euthanized in lieu of anticipated prolonged clinical or surgical protocols which would be necessary to restore them to a plane of physiological normalcy.

Tuberculin testing using old tuberculin from the ARS source was conducted on the entire colony during the months of February and March. The volume of intrapalpebrally administered inoculum was 0.1 ml containing 150 mg OT. This level is considerably above the currently recommended minimum level for tuberculosis detection. On the basis of test readings at 24, 48 and 72 hours post inoculation, all monkeys were shown to be tuberculosis free.

With the exception of some of the animals in the growth and biorhythm study, all of the non-human primates at the Berkeley facility are presently housed in the recently completed animal quarters within the Environmental Physiology Laboratory.

Table 1

Standard Chemical Mixture (SCM)

(Five ml of this true solution contains the amount of the nine different elements found in one gram of Purina monkey pellet).

Compound (Analytical Grade)	Molecular Weight	Compound Required/liter Weight (g)	Compound/liter (mmole)	Element/liter for Compound (mmole)	Element in Standard Chemical Mixture (mM)
$CaCO_3$	100.09	4.9044	49.0	49.0 Ca	49.0 Ca
KH_2PO_4	136.09	3.8377	28.2	28.2 K	28.2 K
				28.2 P	35.4 P
Na_2HPO_4	141.97	1.0222	7.2	7.2 P	
				14.4 Na	20.8 Na
NaCl	58.45	0.3741	6.4	6.4 Na	
				6.4 Cl	15.4 Cl
RbCl	120.94	1.0885	9.0	9.0 Cl	
				9.0 Rb	9.0 Rb
$MgSO_4$	120.39	1.1076	9.2	9.2 Mg	9.2 Mg
				9.2 S	9.8 S
$FeSO_4 \cdot 7H_2O$	278.03	0.1668	0.6	0.6 S	
				0.6 Fe	0.6 Fe
NH_2CONH_2 (urea)	60.06	14.5946	243.0	486.0 N	486.0 N
	Total	27.0959			
$H_3C_6H_5O_7 \cdot H_2O$ (citric acid)	210.14	27.3182	130.0	(Added to dissolve all compounds - pH about 3)	

Note: Urea and $FeSO_4 \cdot 7H_2O$ were dried in a vacuum desiccator; all other compounds were dried overnight in an oven at 80°C and cooled before weighing on an analytical balance and subsequently dissolved to make a total of 1000 ml in a one liter volumetric flask.

Table 2

Analysis of Bovine Liver[1] and SCM[2]

(micrograms per gram)

Element	NBS	EPL[3]		EPL[4]		SCM % Recovery[5]	
		Alkaline Ashing	Kjeldahl	Alkaline Ashing	Kjeldahl	Alkaline Ashing	Kjeldahl
Calcium	(123)	52.7		50.4		98	
Magnesium	(605)	595	561	618	580	97	97
Sodium	2430 ± 130	2570		2300	2270	96	100
Potassium	9700 ± 600		8960		11052		99
Iron	270 ± 20	245		258		102	
Nitrogen	106000 ± 6000		102750		99225		94
Chlorine	(2600)	2644		2687		96	
Phosphorus	Not available	11544	11259	11402	11749	97	94
Sulfur	Not available	?		?		102[6]	

[1] National Bureau of Standards. Standard Reference Material 1577, Bovine Liver. Freeze dried before analysis. Values within parentheses are not certified but for information only.

[2] Standard Chemical Mixture (see Table 1).

[3] Analytical results obtained according to routine procedures used in this laboratory.

[4] Analytical results on a repeated separate analysis.

[5] Analyzed simultaneously with (4). (Except for calcium and sulfur, the per cent recovery also reflects the recovery from a combined mixture of SCM and liver.)

[6] Using empirical procedure. More work is needed to establish reliability.

Table 3

O_2 Consumption and CO_2 Production of an Ethyl Alcohol Lamp.

O_2 Consumption (liters/hour)

	18 Jul	19 Jul	19 Jul	20 Jul	20 Jul	21 Jul	21 Jul	Mean
Predicted	9.72	9.18	9.90	9.00	9.00	8.04	7.86	8.96
Measured	9.06	9.00	9.60	9.30	9.48	8.34	8.28	9.01

CO_2 Production (liters/hour)

	18 Jul	19 Jul	19 Jul	20 Jul	20 Jul	21 Jul	21 Jul	Mean
Predicted	6.48	6.12	6.60	6.00	6.00	5.34	5.22	5.97
Measured	6.24	6.12	6.60	6.12	6.18	5.46	5.40	6.02

Respiratory Quotient (CO_2 Production/O_2 Consumption)

	18 Jul	19 Jul	19 Jul	20 Jul	20 Jul	21 Jul	21 Jul	Mean
Predicted	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67
Measured	0.69	0.68	0.69	0.66	0.65	0.65	0.65	0.67

Table 4

Mean values in grams N/day urine nitrogen, feces nitrogen and integumental nitrogen losses for 6 adult pig-tailed monkeys at different levels of dietary nitrogen intake.
Values in parentheses are extrapolated or interpolated.

Diet Intake (N_d)	Urine Loss (N_u)	Feces Loss (N_f)	Integumental Loss (N_i)	($N_u+N_f+N_i$)	$N_d-(N_u+N_f+N_i)$
(0)	(0.37)	(0.23)	(0.20)	(0.80)	(-0.80)
0.15	0.42	0.22	0.20	0.84	-0.69
0.30	0.48	0.21	0.20	0.89	-0.59
0.60	0.59	0.19	0.20	0.98	-0.38
0.80	0.68	0.14	0.20	1.02	-0.22
0.90	0.66	0.16	0.20	1.02	-0.12
1.00	0.64	0.23	0.20	1.07	-0.07
1.10	0.72	0.21	0.20	1.13	-0.03
(1.14)	(0.73)	(0.21)	(0.20)	(1.14)	(0)
1.20	0.75	0.21	0.20	1.16	0.04
1.50	0.79	0.32	0.20	1.31	0.19
1.80	0.81	0.32	0.20	1.33	0.47
2.10	0.81	0.36	0.20	1.37	0.73
2.40	1.01	0.17	0.20	1.38	1.02
2.55	1.44	0.22	0.20	1.86	0.69
2.70	1.28	0.32	0.20	1.80	0.90

Table 5

Daily nitrogen loss in grams N/day, exclusive of urine, feces and gases, in 6 pig-tailed monkeys on Diet 4.

Day	Monkey Number 174	175	296	321	390	395
1	0.17	0.15	0.24	0.36	0.17	0.22
2	0.10	0.14	0.14	0.05	0.12	0.15
3	0.20	0.09	0.10	0.21	0.39	0.39
4	0.13	0.11	0.14	0.22	0.15	0.11
5	0.10	0.10	0.10	0.10	0.11	0.08
6	0.14	0.13	0.09	0.20	0.19	0.19
7	0.15	0.18	0.13	0.21	0.17	0.12
8	0.24	0.22	0.15	0.27	0.11	0.12
9	0.16	0.19	0.23	0.08	0.09	0.16
10	0.09	0.08	0.10	0.12	0.09	0.10
11	0.13	0.19	0.12	0.20	0.14	0.17
12	0.15	0.22	0.17	0.22	0.20	0.14
13	0.18	0.19	0.13	0.09	0.21	0.11
14	0.11	0.11	0.08	0.22	0.15	0.07
Mean	0.146	0.150	0.137	0.182	0.164	0.152
$\pm$ S.D.	0.042	0.048	0.048	0.085	0.076	0.080

Mean $\pm$ S.D. of all values = 0.155 $\pm$ 0.065 g N/day.

Table 6

Recommended daily protein allowance for the adult male pig-tailed monkey *(Macaca nemestrina)*.

	Whole Egg		Protein Mixture[1]	
	protein	nitrogen	protein	nitrogen
per adult animal[2]				
Recommended daily allowance, g	14.0	2.2	20.0	3.2
per kg of body weight				
Recommended daily allowance, g	1.4	0.2	2.0	0.3
as percentage of total calories in diet				
g per 100 kcal	2.0	0.3	3.0	0.5

[1] Protein of 70% utilization value. 70% is used by the U.S. National Research Council in recommending protein intake for man, when the protein source is a mixture of less than optimal value.

[2] For an average adult animal weighing approximately 10 kg.

Table 7

Daily Food and Water Consumption During an 18-Day Metabolic Balance Trial

Trial No.	Monkey No.	Cage Days 1-6		Pod Days 7-12		Cage Days 13-18	
		Food per day (g)	Water per day (ml)	Food per day (g)	Water per day (ml)	Food per day (g)	Water per day (ml)
1	307	200	900	176	775	191	900
2	314	151	900	131	900	118	900
3	411	120	610	68	388	115	628
4	405	144	720	130	463	131	649
5	314	150	892	128	862	146	892
Mean		153	804	126	678	140	793
SD		± 29	±133	± 38	±236	± 31	±142
SE of Mean		± 13	± 60	± 17	±106	± 14	± 63

Table 8

O_2 Consumption and CO_2 Production of a Male Pig-tailed Monkey #314, Pompey

		24 Jul	25 Jul	26 Jul	27 Jul	Mean
O_2 Consumption (liters/hour)		3.66	3.57	3.56	3.67	3.62
CO_2 Production (liters/hour)		3.17	3.02	3.22	3.27	3.17
Respiratory Quotient		0.87	0.85	0.90	0.89	0.88
F_{O_2}	Room Air	0.2078	0.2078	0.2078	0.2078	0.2078
	Pod Air	0.2004	0.2004	0.2006	0.2004	0.2005
	ΔF_{O_2}	0.0074	0.0074	0.0072	0.0074	0.0073
F_{CO_2}	Room Air	0.0003	0.0003	0.0003	0.0003	0.0003
	Pod Air	0.0067	0.0066	0.0068	0.0069	0.0068
	ΔF_{CO_2}	0.0064	0.0063	0.0065	0.0066	0.0065
F_{H_2O}	Room Air	0.0131	0.0132	0.0128	0.0130	0.0130
	Pod Air	0.0213	0.0200	0.0200	0.0189	0.0200
	ΔF_{H_2O}	0.0082	0.0068	0.0072	0.0059	0.0070
T (°C)	Room Air	22.5	22.4	22.4	22.0	22.3
	Pod Air	26.5	26.5	26.5	26.5	26.5
	ΔT	4.0	4.1	4.1	4.5	4.2
Pod Air Flow Rate (Liters/min)		8.25	8.00	8.25	8.25	8.18

Table 9

Final Rectal Temperatures for Four Monkeys for Air Temperatures from 5° to 40°C at 50% Relative Humidity.

Subject	Air Temperature (°C)							
	5	10	15	20	25	30	35	40
#378	33.0	35.6	37.4	--	38.2	--	39.0	41.5
#405	37.5	37.6	38.0	38.0	38.0	38.5	39.1	40.0
#377	29.5	32.1	34.6	35.2	37.5	38.0	38.8	42.0*
#408	36.1	37.0	37.5	37.4	37.8	39.6	41.5*	42.0*

Data enclosed in boxes indicate air temperatures at which each subject was in thermal equilibrium defined as no more than 0.1°C change in rectal temperature during final 80 minutes of 260 minute exposure.

* These tests terminated before 260 minutes due to high rectal temperature. Temperature indicated is final temperature just prior to termination of test.

Table 10

Change in Heart Rate (beats/min) Expressed as Final Rate at Air Temperature Indicated Minus Final Rate During Control (23°C).

Subject	Air Temperature (°C)							
	5	10	15	20	25	30	35	40
#378	-15	+15	+30	--	-3	--	+33	+51
#405	+27	+6	-3	-3	+3	-9	+21	+39
#377	-72	-36	-12	-33	+6	+12	+6	+69*
#408	+24	+36	+36	-3	-3	+81	+83*	+78*

Data enclosed in boxes indicate air temperatures at which each subject was in thermal equilibrium.

* These tests terminated before 260 minutes due to high rectal temperature. The heart rate indicated is final rate just prior to termination of test.

Table 11

Number of Days for Restabilization of Quartile Crossing Points Following a 180° L-L shift.

	#364 (pinealectomized)				#345 (control)				#412 (control)			
	1↑	2↑	1↓	2↓	1↑	2↑	1↓	2↓	1↑	2↑	1↓	2↓
Shift #1:	7	1	9	9	1	2	8	5	1	6	6	8
Shift #2:	8	5	11	7	4	1	7	7	6	7	4	7
Shift #3:	1	1	9	8	4	1	6	6	1	1	4	4

1↑ : time of one quartile rise.

2↑ : " " two quartiles rise (mean).

1↓ : " " one quartile fall.

2↓ : " " two quartiles fall (mean).

Table 12

Changes in Body Mass Associated with Age in the Male Pig-tailed Monkey.

Age in Years	(n)	Body Mass in kg Mean	S.D.
Birth	10	0.67	0.08
0.5	8	1.18	0.10
1.0	8	1.83	0.22
1.5	6	2.55	0.08
2.0	6	2.90	0.13
2.5	6	3.17	0.13
3.0	5	3.42	0.14
3.5	4	3.67	0.16
4.0	4	4.18	0.30
4.5	4	5.09	0.62
5.0	2	6.25	0.64
5.5	2	7.05	0.50
6.0	2	7.89	0.36
6.5	2	8.16	0.30
7.0	2	8.52	0.28
7.5	2	8.83	0.25
8.0	2	8.99	0.28

Table 13

Male Pig-tailed Monkeys
Order of Appearance of Ossification Centers

Time Period	Ossification Center	Mean Age at Appearance (mos.)	Growth Curve Phase
Present at Birth	* Humerus, proximal		
	Humerus, distal		
	Radius, distal		
	Ulna, distal		
	Carpals		
	Metacarpals		
	Phalanges, proximal row (hand)		
	Femur, proximal		
	* Femur, distal		
	* Tibia, proximal		
	Tibia, distal		
	Fibula, distal		
	* Tarsals		
	Metatarsals		
	Phalanges, proximal row (foot)		
1 to 12 months	Humerus, medial epicondyle	0.4	I
	Radius, proximal	2.2	I
	Ulna, proximal	5.2	I
	Femur, lesser trochanter	10.4	I
12 to 24 months	Patellar	13.8	II
	Fibula, proximal	22.3	III
	Calcaneal	23.8	III
24 to 36 months	Sesamoids, 1st digit, proximal (foot)	28.4	III
	Sesamoids, 1st digital (hand)	35.4	III
	Sesamoids, 1st digit distal (foot)	35.7	III
36 to 48 months	Sesamoids, radial carpal	45.5	IV
48 to 60 months	Fabellar	48.5	IV
	Tibia, tuberosity	52.0	IV
	Sesamoids, lateral, tarsal	52.3	IV
	Sesamoids, 2-4th digital (foot)	58.5	V
60 months +	Sesamoids, medial, tarsal	65.0	V
	Sesamoids, 5th digit (foot)	65.5	V

* Present at birth in human male.

Table 14

Male Pig-tailed Monkeys
Order of Completion of Epiphyseal Fusion

Time Period	Epiphysis	Mean Age in Mos.	Growth Curve Phase
36 to 48 months	Os Coxae, acetabular	40.5	End of III
	Humerus, distal	46.3	IV
48 to 60 months	Femur, proximal	57.0	V
	Humerus, medial epicondyle	60.0	V
60 to 72 months	Femur, lesser trochanter	70.0	V
72 to 84 months	Ulna, proximal	77.0	V
	Phalanges, proximal row (foot)	77.0	V
	Calcaneal	78.0	V
	Radius, proximal	78.5	V
84 to 96 months	Metatarsal	84.5	V
	Metacarpal	87.5	V
	Phalanges, proximal row (hand)	87.5	V
	Femur, distal	87.5	V
	Fibula, proximal	87.5	V
96 to 108 months	Tibia, proximal	91.5	V
	Tibia, distal	91.5	V
	Fibula, distal	91.5	V
	Humerus, proximal	>97	V
	Radius, distal	>97	V
	Ulna, distal	>97	V
	Tibia, tuberosity	>97	V

Table 15

Post-Natal Appearance of Ossification Centers in the Male Pig-tailed Monkey with Comparative Data for the Male Human

Ossification Center (epiphysis)	Age (Months)								Whole Number Ratio Human Mos. / Monkey Mos.
	Male Pig-tailed Monkey						Human Male**		
	n	Mean	SD	SE	Range	Coef. of Var.	Mean	Range	
Humerus, proximal	12	B*						B-3	1-3
Humerus, medial epicondyle	11	0.4	0.7	0.2	B-2	153.2	84	60-84	44-60
Humerus, distal	12	B					5	1.5-8	1.5-8
Radius, proximal	11	2.2	0.9	0.3	1-4	40.0	60	36-72	18-36
Radius, distal	12	B					12	3-18	3-18
Ulna, proximal	10	5.2	2.2	0.7	2-9	43.0	120		23
Ulna, distal	12	B					72	48-108	48-108
Carpals	12	B					6	0-18	1-18
Metacarpals	12	B					15	10-24	10-24
Phalanges, proximal row	12	B						5-30	5-30
Sesamoids, radial carpal	4	45.5	3.7	1.8	42-50	8.1			
Sesamoids, 1st digital	5	35.4	8.0	3.6	28-46	22.7			
Femur, proximal	12	B					4	2-8	2-8
Femur, lesser trochanter	8	10.4	2.2	0.8	8-14	21.2	144	120-156	11-15
Femur, distal	12	B					B		1
Patellar	8	13.8	1.5	0.5	12-16	10.8		48-60	4
Fabellar	4	48.5	3.1	1.5	45-52	6.4			
Tibia, proximal	12	B					B		1
Tibia, tuberosity	3	52.0	7.0	4.0	45-59	13.4		84-180	2-3
Tibia, distal	12	B					6	3-18	3-18
Fibula, proximal	6	22.3	2.9	1.2	18-26	12.9	48	24-66	2-3
Fibula, distal	12	B					12	6-24	6-24
Tarsals	12	B					B		1
Calcaneal	6	23.8	4.9	2.0	18-31	20.6		60-144	3-5
Metatarsals	12	B					24	6-48	6-48
Phalanges, proximal row	12	B						6-30	6-30
Sesamoids, lateral-tarsal	3	52.3	8.3	4.8	43-59	15.9			
Sesamoids, medial-tarsal	2	65.0	4.2	3.0	62-68	6.5			
Sesamoids, 1st digit proximal	5	28.4	7.0	3.1	23-40	24.7			
Sesamoids, 1st digit distal	4	35.7	11.7	5.9	24-49	32.5			
Sesamoids, 2-4th digital	2	58.5	17.7	12.5	46-71	30.2			
Sesamoids, 5th digital	2	65.5	14.8	10.5	55-76	22.8			

* B = probably present at birth.

** Skeletal Maturation Chart of R. Hugo MacKay from Eastman Kodak Company Medical Division, Rochester 4, N.Y.

Table 16

Times of Complete Epiphyseal Fusion in the Male Pig-tailed Monkey with Comparative Data for the Male Human

Epiphysis	Age (months								Whole Number Ratio Human Mos. / Monkey Mos.
	Male Pig-tailed Monkey						Male Human*		
	n	Mean	SD	SE	Range	Coef. of Var.	Mean	Range	
Humerus, proximal	2	>97						228-252	2
Humerus, medial epicondyle	2	60.0	7.1	5.0	55-65	11.8	216		3
Humerus, distal	3	46.3	5.5	3.2	40-50	11.9	204		4
Radius, proximal	2	78.5	6.4	4.5	74-83	8.1		180-204	2-3
Radius, distal	2	>97					228		2
Ulna, proximal	2	77.0	8.5	6.0	71-83	11.0		180-204	2-3
Ulna, distal	2	>97					228		2
Metacarpal	2	87.5	6.4	4.5	83-92	7.3		168-252	2-3
Phalanges, proximal row	2	87.5	6.4	4.5	83-92	7.3		168-252	2-3
Os coxae, acetabular	4	40.5	2.4	1.2	39-44	5.9		Puberty	
Femur, proximal	2	57.0	11.3	8.0	49-65	20.0		204-216	4
Femur, lesser trochanteric	2	70.0	14.1	10.0	60-80	20.2		192-204	3
Femur, distal	2	87.5	6.4	4.5	83-92	7.3		216-228	2-3
Tibia, proximal	2	91.5	0.7	0.5	91-92	0.8		216-228	2
Tibia, tuberosity	2	>97					228		2
Tibia, distal	2	91.5	0.7	0.5	91-92	0.8	216		2
Fibula, proximal	2	87.5	6.4	4.5	83-92	7.3		216-240	2-3
Fibula, distal	2	91.5	0.7	0.5	91-92	0.8	216		2
Calcaneal	2	78.0	1.4	1.0	77-79	1.8		144-264	2-3
Metatarsal	2	84.5	2.1	1.5	83-86	2.5		168-252	2-3
Phalanges, proximal row	2	77.0	8.5	6.0	71-83	11.0	216	132-264	2-3

* Skeletal Maturation Chart of R. Hugo MacKay from Eastman Kodak Co. Medical Division, Rochester 4, N.Y.

Table 17

Changes in Body Mass Associated with Age in Female Pig-tailed Monkey

Age in Years	(n)	Body Mass in kg Mean	S.D.
Birth	10	0.61	0.09
0.5	8	1.14	0.10
1.0	8	1.86	0.16
1.5	7	2.35	0.22
2.0	7	2.77	0.23
2.5	7	3.24	0.26
3.0	7	3.66	0.26
3.5	6	4.01	0.29
4.0	6	4.32	0.26
4.5	4	4.66	0.28
5.0	4	4.94	0.30
5.5	3	5.22	0.42
6.0	3	5.49	0.49
6.5	2	5.85	0.63
7.0	2	6.00	0.63

Table 18

Female Pig-tailed Monkeys
Order of Appearance of Ossification Centers

Time Period	Ossification Center	Mean Age at Appearance (mos.)	Difference from Males in Months
Present at Birth	*Humerus, proximal		
	Humerus, distal		
	Radius, distal		
	Ulna, distal		
	Carpals		
	Metacarpals		
	Phalanges, proximal row (hand)		
	Femur, proximal		
	*Femur, distal		
	*Tibia, proximal		
	Tibia, distal		
	Fibula, distal		
	*Tarsals		
	Metatarsals		
	Phalanges, proximal row (foot)		
1 to 12 months	Humerus, medial epicondyle	<2	?
	Radius, proximal	<2	1
	Ulna, proximal	<2	3
	Femur, lesser trochanter	8.3	2.1
	Patellar	9.3	4.5
12 to 24 months	Fibula, proximal	13.1	9.2
	Calcaneal	14.7	9.1
	Sesamoids, 1st digit proximal (foot)	18.6	9.8
	Sesamoids, 1st digit (hand)	20.2	15.2
	Sesamoids, radial carpal	22.3	13.3
	Fabellar	23.3	25.2
24 to 36 months	Sesamoids, 1st digit, distal (foot)	24.7	11.0
	Tibia, tuberosity	25.9	26.1
	Sesamoids, lateral tarsal	28.7	23.6
	Sesamoids, 2-4th digital	31.0	27.5
36 to 48 months	Sesamoids, 5th digital	41.2	18.8
	Sesamoids, medial, tarsal	46.7	18.3

* Present at birth in human female.

Table 19

Female Pig-tailed Monkeys
Order of Completion of Epiphyseal Fusion

Time Period	Epiphysis	Mean Age in Mos.	Difference from Males in Months
24 to 36 months	Os coxae, acetabular	27.0	13.5
	Humerus, distal	31.7	14.6
36 to 48 months	Femur, proximal	37.0	20.0
	Humerus, medial epicondyle	38.6	21.4
	Femur, lesser trochanter	41.7	28.3
	Tibia, tuberosity	47.0	50.0
	Radius, proximal	47.7	30.8
48 to 60 months	Ulna, proximal	48.3	28.7
	Phalanges proximal row (hand)	51.7	35.8
	Calcaneal	51.7	26.3
	Metatarsal	55.7	28.8
	Phalanges, proximal row (foot)	55.7	21.3
	Femur, distal	56.0	31.5
	Metacarpal	56.0	31.5
	Tibia, distal	56.0	35.5
	Tibia, proximal	58.5	33.0
	Humerus, proximal	59.0	38.0
60 to 72 months	Fibula, proximal	66.0	21.5
72 to 84 months	Radius, distal	75.0	22.0
	Ulna, distal	75.0	22.0
	Fibula, distal	>75.0	22.0

Table 20

Post-natal Appearance of Ossification Centers in the Female Pig-tailed Monkey with Comparative Data for the Female Human

Ossification Center (epiphysis)	Age (Months)								Whole Number Ratio Human Mos. / Monkey Mos.
	Female Pig-tailed Monkey						Female Human**		
	n	Mean	SD	SE	Range	Coef. of Var.	Mean	Range	
Humerus, proximal	9	B*						B-3	1-3
Humerus, medial epicondyle	8	<2					60	36-72	
Humerus, distal	9	B					4	1-6	1-6
Radius, proximal	8	<2					48	36-72	
Radius, distal	9	B					12	3-18	3-18
Ulna, proximal	8	<2					96		
Ulna, distal	9	B					60	48-108	48-108
Carpals	9	B					6	B-18	1-18
Metacarpals	9	B					15	10-24	10-24
Phalanges, proximal row	9	B						5-30	5-30
Sesamoids, radial carpal	6	22.3	1.2	0.5	21-24	5.4			
Sesamoids, 1st digital	6	20.2	2.9	1.2	15-23	14.2			
Femur, proximal	9	B					4	1.5-6	1.5-6
Femur, lesser trochanter	7	8.3	2.0	0.7	5-11	23.6	132	108-144	13-20
Femur, distal	9	B					B		1
Patellar	7	9.3	2.1	0.8	8-13	23.0	36		3-4
Fabellar	7	23.3	3.0	1.1	21-29	12.9			
Tibia, proximal	9	B					B		1
Tibia, tuberosity	7	25.9	3.3	1.2	21-29	12.7		84-180	3-6
Tibia, distal	9	B					6	3-18	3-18
Fibula, proximal	7	13.1	0.7	0.3	12-14	5.7	36	24-66	2-5
Fibula, distal	9	B					9	6-24	6-24
Tarsals	9	B					B		
Calcaneal	7	14.7	2.1		13-18	14.0		60-144	4-8
Metatarsals	9	B					24	6-48	6-48
Phalanges, proximal row	9	B						6-30	6-30
Sesamoids, lateral-tarsal	7	28.7	4.8	1.8	25-39	16.8			
Sesamoids, medial-tarsal	4	46.7	6.4	3.2	42-56	13.7			
Sesamoids, 1st digit proximal	7	18.6	2.2	0.8	15-21	11.9			
Sesamoids, 1st digit distal	7	24.7	6.1	2.3	16-34	24.6			
Sesamoids, 2-4th digital	4	31.0	10.7	5.3	20-44	34.3			
Sesamoids, 5th digital	4	41.2	5.9	2.9	33-46	14.3			

* B = probably present at birth.

** Skeletal Maturation Chart of R. Hugo MacKay from Eastman Kodak Company Medical Division, Rochester 4, N.Y.

Table 21

Times of Complete Epiphyseal Fusion in the Female Pig-tailed Monkey with Comparative Data for the Female Human

Epiphysis	Age (Months)								Whole Number Ratio
	Female Pig-tailed Monkey						Female Human*		
	n	Mean	SD	SE	Range	Coef. of Var.	Mean	Range	Human Mos. / Monkey Mos.
Humerus, proximal	2	59.0	4.2	3.0	56-62	7.2		216-240	4
Humerus, medial epicondyle	6	38.6	2.8	1.2	36-42	7.2	180		5
Humerus, distal	6	31.7	10.5	4.3	25-52	33.2	168		5
Radius, proximal	4	47.7	9.5	4.7	36-58	19.8		168-180	3-5
Radius, distal	1	75.0					204		3
Ulna, proximal	3	48.3	8.6	5.0	39-56	17.8		168-180	3-4
Ulna, distal	1	75.0					204		3
Metacarpal	3	56.0	10.6	6.1	48-68	18.9		168-252	3-4
Phalanges, proximal row	3	51.7	3.5	2.0	48-52	6.8		168-252	3-5
Os coxae, acetabular	6	27.0	4.5	1.8	19-32	16.7		Puberty	
Femur, proximal	5	37.0	11.5	5.2	25-55	31.2		192-204	4-8
Femur, lesser trochanteric	4	41.7	7.1	3.5	37-52	16.9		192-204	4-5
Femur, distal	4	56.0	6.4	3.2	47-62	11.2	204		4
Tibia, proximal	2	58.5	7.8	5.5	53-62	13.3		192-216	3-4
Tibia, tuberosity	1	47.0					228		5
Tibia, distal	1	56.0					216		4
Fibula, proximal	2	66.0	2.8	2.0	64-68	4.3		192-216	3
Fibula, distal	1	>75					192		2-3
Calcaneal	3	51.7	8.5	4.9	43-60	16.4		144-264	3-4
Metatarsal	3	55.7	2.5	1.4	53-58	4.5		168-252	3-4
Phalanges, proximal row	3	55.7	2.1	1.2	54-58	3.7		132-264	2-5

* Skeletal Maturation Chart of R. Hugo MacKay from Eastman Kodak Co. Medical Division, Rochester 4, N.Y.

Table 22

Chronological Results of Post-Surgical Examination of Vascular Catheters Implanted in the Pig-tailed Monkey #399, Catesby

Day Post-Surgery of Catheter Checks	Aorta		Pulmonary Artery		Body Mass (kg)
	Condition	Sol'n Used to Fill Catheter After Patency Check	Condition	Sol'n Used to Fill Catheter After Patency Check	
1 through 8	W	H	W	H	
12	I-W	H	W	H	
13	W	H	W	H	
15	N-W	B	W	H	
18	W	H	W	H	
21 through 42	W	H	W	H	
47	W	H	I-W	H	
50 through 85	W	H	W	H	
88	I-W	H	W	H	10.20
92	I-W	H	W	H	
95 through 161	W	H	W	H	
165	W	H	I-W	H	
168 through 204	W	H	W	H	
214	W	H	NW	B	
216	W	H	I-W	H	
221 through 290	W	H	W	H	
297	W	H	I-W	H	
300	I-W	B	I-W	B	
304 through 325	W	H	W	H	
325	W	H	NW	B	
339 through 362	W	H	W	H	
367	I-W	B	I-W	B	10.96
370 through 388	W	H	W	H	
393	I-W	B	I-W	B	11.00
396	I-W	B	I-W	B	
401	W	B	W	B	
404	W	B	W	B	
409	W	H	W	H	
413	W	H	I-W	B	10.96
416	I-W	B	W	H	
420	W	H	I-W	B	
423 to 430	W	H	W	H	10.93

Legend:

Solution

H = Heparin saline

B = Brinase saline

Condition

W = Withdrawal

I-W = Slight injection before withdrawal

NW = Non-functional

Table 23

Chronological Results of Post-Surgical Examination of Vascular Catheters Implanted in the Pig-tailed Monkey #404, Tyrrel

Days Post-Surgery of Catheter Checks	Aorta		Pulmonary Artery (Dacron Felt Patch)		
	Condition	Sol'n Used to Fill Catheter After Patency Check	Condition	Sol'n Used to Fill Catheter After Patency Check	Body Mass (kg)
1 through 21	W	H	W	H	8.80
25 through 65	W	H	W	H	Series of LBNP tests.
68 through 103	W	H	W	H	Bioinstrumentation tests.
107 through 127	W	H	W	H	8.60
130 through 205	W	H	W	H	8.70
208	I-W	H	W	H	
209 through 230	W	H	W	H	
233	I-W	B	W	H	8.90
234 through 253	W	H	W	H	
257	W	H	I-W	B	
260	NW	B	W	H	
264	NW	B	W	H	
267	NW	B	W	H	
271	cath. excised at skin line		W	H	
278			I-W	H	
281 through 337			W	H	8.45
341 through 396			W	H	In isolation box intermittently.
400			I-W	B	Catheter accidentally cut, then spliced.
404 through 458			W	H	
462			I-W	B	Leak in splice repaired under anesthesia.
466 through 499			W	H	8.74
504 through 510			W	H	8.95
513 through 517			W	H	8.86
520 through 541			W	H	9.23
545			I-W	B	9.07
548			W	H	9.04

Legend:

Solution

H = Heparin saline

B = Brinase saline

Condition

W = Withdrawal

I-W = Slight injection before withdrawal

NW = Non-functional

Table 24

Census of Non-Human Primates *(Macaca nemestrina)*

1 February to 31 July 1972

	Feb	Mar	Apr	May	Jun	Jul
No. at start of month	56	54	55	55	55	53
Acquisitions	0	1*	0	0	0	0
Deaths	0	0	0	0	0	0
Sacrifices	2	0	0	0	2	0
No. at end of month	54	55	55	55	53	53

* Birth, on 19 March 1972, Male #418, Robin < #51 Voltimand / #261 Bona